REVERSE MICELLE EXTRACTION OF ERYTHROMYCIN WITH MIXED SURFACTANT ANIONIC AND ZWITTERIONIC

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DEDICATION

Dedicated to my beloved husband, Manzurudin, and to my sons, Zayyan, Zareef and Zayn and my parents

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ABSTRAK

Kebanyakan kajian mengenai misel terbalik dijalankan dengan menggunakan surfaktan anion iaitu garam natrium bis(2-etilheksil) sulfosuksinat (AOT). Walaubagaimanapun, molekul yang terperangkap didalam misel terbalik AOT dilaporkan terjejas disebabkan oleh interaksi elektrostatik yang kuat dan penggunaan surfaktan yang tinggi. Oleh itu, penambahan surfaktan SB3-12 zwiterion kepada misel terbalik AOT dicadangkan untuk pengekstrakan eritromisin. Tujuan kajian ini adalah untuk memerhatikan kestabilan campuran misel yang terbentuk berdasarkan parameter termodinamik yang boleh menjadi kaedah berpotensi bagi pengekstrakan antibiotik. Parameter kinetik dalam pemindahan kehadapan diselidik, di mana pemindahan jisim kinetik, mekanisme penyerapan dan juga optimum eritromisin yang diekstrak telah ditentukan. Data eksperimen dianalisis dengan menggunakan model teori dua selaput, isoterma Langmuir, Freundlich dan Sips dan model yang terbaik telah ditentukan dengan menggunakan analisis ralat. Untuk mengoptimumkan perpindahan kehadapan, reka bentuk uji kaji sistematik termasuk satu-faktor-pada-satu masa (OFAT) dan reka bentuk faktorial sepenuhnya digunakan dalam proses penyaringan awal untuk menentukan faktor-faktor pembolehubah yang penting. Keadaan optimum dalam pemindahan kehadapan kemudiannya digunakan untuk proses selanjutnya di dalam pengekstrakan kebelakang. Faktor yang mempengaruhi pengekstrakan kebelakang dan pemindahan jisim kinetik kemudiannya telah disiasat dan akhirnya keadaan yang optimum telah diperolehi. Nilai CMC_{mix} diperolehi adalah rendah diantara 0.7-5.7 g/L menunjukkan bahawa aktiviti permukaan unggul bagi campuran berbanding dengan surfaktan tunggal. Nilai negatif ΔG_m, ΔG_{ads} and ΔG^o_{ex} menunjukkan bahawa proses penjerapan berlaku secara spontan dan campuran misel terbentuk secara stabil dari segi termodinamik. Hasil membuktikan bahawa penambahan SB3-12 meningkatkan kestabilan campuran misel yang kemudiannya menyediakan persekitaran mikro yang lebih baik untuk biomolekul. Teori dua selaput adalah sesuai untuk pemindahan jisim eritromisin kehadapan dan didapati dikawal oleh pelarutan antara muka dan penyerapan erithromisin dalam lapisan sempadan fasa akueus. Isoterma terbaik untuk pemindahan erithromisin adalah isoterma Langmuir, yang menunjukkan nilai pekali penentuan tertinggi dan disahkan oleh tiga jenis analisis ralat. Hasil FFD menunjukkan bahawa kepekatan AOT, pecahan zwiterion dan pH fasa akueus, adalah faktor terpenting di dalam pemindahan kehadapan. Nilai optimum yang diperoleh untuk pemindahan kehadapan ialah kepekatan AOT, 80.7 g/L; pecahan zwiterion, 0.24; dan pH fasa akueus, 4.7 dengan pemindahan eritromisin optimum adalah 95.70%. Dapat disimpulkan bahawa keterlarutan eritromisin yang tinggi berjaya diperolehi walaupun dengan kepekatan AOT yang rendah mencerminkan sinergi antara AOT dan SB3-12. Bagi pengekstrakan ke belakang, kadar pengekstrakan pada umumnya dua kali lebih perlahan, bagaimanapun, masa keseimbangan didapati lebih cepat daripada kaedah konvensional yang telah dilaporkan sebelum ini. Keadaan optimum bagi pengekstrakan kebelakang adalah isopropanol v/v, 3.9 %; kepekatan NaCl, 26.5 g/L; dan pH akueus 8.4 untuk berjaya mendapatkan pengekstrakan eritromisin 95.01%. Dalam pengekstrakan kebelakang, pH juga didapati sebagai parameter penting yang mengalakkan pemindahan kebelakang kerana erithromycin mudah dibebaskan dari misel terbalik. Eritromisin mendominasi pada pH yang lebih tinggi disebabkan penolakkan dengan surfaktan yang mengalakkan pemindahan kebelakang.

ABSTRACT

Most of the studies on reverse micelle extraction have been performed by using single anionic surfactant bis(2-ethylhexyl) sulfosuccinate sodium salt (AOT). However, the bio-molecules hosted in AOT reverse micelle were reported to be negatively affected by strong electrostatic interactions and high consumption of surfactant needed. Therefore, the addition of zwitterionic SB3-12 surfactant to the AOT reverse micellar was proposed for the extraction of erythromycin. This study aimed to observe the stability of mixed micelle formed based on the thermodynamic parameters which can be a potential method for antibiotic extraction. The kinetic parameter in the forward transfer was investigated, where the mass transfer kinetic, adsorptions mechanisms as well as optimum erythromycin extracted were determined. The experimental data were analyzed using Two-film theory, Langmuir, Freundlich and Sips isotherm model and the best fitted isotherm model was then determined using error analysis. For the optimization of forward extraction, a systematic experimental design including One-factor-at-time (OFAT) and full factorial design was used in the initial screening process to determine the significant variables factors. The optimized condition in forward extraction was further used in backward extraction. The factor effecting of backward extraction and kinetic mass transfer during recovery have been investigated. The CMC_{mix} show a lower value in the range of 0.7-5.7 g/L suggests a superior surface activity compared to single surfactant. The negative value of ΔG_m, ΔG_{ads} and ΔG^o_{ex} indicate that the adsorption process was spontaneous and mixed reverse micelle formed was thermodynamically stable. The result proved that the addition of SB3-12 increase the stability of mixed micelle formed which provided a better microenvironment for bio-molecules. The two-film theory is appropriate for the mass transfer kinetic of erythromycin in forward extraction and the mass transfer kinetic was found to be controlled by interface solubilisation and the diffusion of the erythromycin in the aqueous phase boundary layer. The best fitting isotherm for erythromycin transfer was Langmuir isotherm, which is demonstrated by the highest values of coefficient of determination and was confirmed by three types of error analysis. The results of full factorial design (FFD) indicated that the AOT concentration, zwitterion fraction and pH of the aqueous phase, are the significant factors in forward extraction. The optimum values obtained for the forward transfers were AOT concentration, 80.7 g/L; zwitterion fraction, 0.24; and pH of aqueous, 4.7 with the optimum erythromycin transfer at 95.70 % was attained. It can be concluded that the highest erythromycin solubilisation was successfully obtained even with a low AOT surfactant which reflects the synergy between AOT and SB3-12. For the backward extraction, the extraction rates generally two orders slower than forward extraction, however, equilibrium time was found to be faster than the conventional method previously reported. The backward optimum conditions namely isopropanol v/v 3.9%; NaCl concentration, 26.5 g/L; and pH of aqueous 8.4 fulfill the conditions to successfully obtain a higher erythromycin recovery (95.01%). In backward extraction, pH was found to be significant which promotes backward extraction since erythromycin was easily to release from the mixed reverse. The anionic erythromycin predominates at higher pH, causes repulsion with the surfactant which promotes backward transfer.

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LIST OF SYMBOLS

%	Percent
mN/m	MilliNewton/metre
nm	Nanometer
°	Degree
mL	Milliliter
rpm	Revolutions per minute
min	Minute
°C	Degree Celsius
g	Gram
L	Liter
M	Molar
µ	Micro
cm	Centimeter
v/v	Volume per volume
N	Normality
K	Kelvin
s	Second
J	Joule

LIST OF ABBREVIATION

RMSS	Reverse micelle solvent system
AOT	Sodium bis(2-ethylhexyl) sulfosuccinate
TWEEN	Polyoxyethylenesorbitan
SB3-12	3-**(***N*,*N*-Dimethyldodecylammonio) propanesulfonate.
UV-VIS	Ultraviolet–visible
NaCl	Sodium chloride
OFAT	One-factor-at-a-time
R_m	Reverse micelle radius
FTIR	Fourier-Transform Infrared
RSM	Response surface methodology
EO	Ethylene oxide
DSP	Downstream processing
GMP	Good manufacturing process
CCC	Counter-current chromatography
CMC	Critical micelle concentration
W_O	Water content
GMO	Non-ionic monoolein
OL	Oleyl lactate
DOLPA	Dioleyl phosphoric acid
pI	Isoelectric point
SSE	Sum of the squares of the error
RMSE	Root-mean-square error
χ^2	Chi-square analysis
DOE	Design of experiment
BBD	Box–Behnken design
ANOVA	Analysis of variance
CTAB	Hexadecyltrimethylammonium bromide
TX100	Octylphenol Ethoxylate

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